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The Use of Copper Sulfate as a Cure for Fish Diseases Caused by Parasitic Dinoflagellates of the Genus *Oodinium*

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(Plate I; Text-figure 1)

Introduction

Coral-reef fishes from the Hawaiian Islands have been received by the Steinhart Aquarium for many years and until 1951 they were relatively free from any disease that could be considered as epidemic. In that year, however, about ten days after an exceptionally large shipment arrived from Honolulu, a gill disease broke out in the tanks. At first the fishes were not suspected of having any specific disease. Many of them congregated near the surface and were observed to be respiring very rapidly, obviously in great distress. It was apparent that either the water was deficient in oxygen or the fishes for some reason were not able to utilize the oxygen that was present. On the assumption that the tanks had become contaminated, they were drained and refilled with fresh sea water and the circulation of water was substantially increased, but the fish were not relieved of their respiratory trouble. As time went by, more individuals congregated near the surface, gasping for air, and it was not long before some of them died.

Microscopic examination revealed innumerable minute, oval, parasitic organisms clinging to the gill filaments of the dead fish (Plate I), so thickly planted that they were obviously interfering with the respiratory function of the gills. They were determined to be a species of *Oodinium,* possibly *ocellatum.* Left overnight in a dish of water, they were re-examined the next morning and it was found that some had divided once and many twice, so that all had passed into the 2- or 4-cell stage of development within the period of about 24 hours. Nigrelli (1936) found that each *Oodinium* organism, after becoming detached from the gills of a fish, settled to the substratum, where it gave rise to palmella stages of 2, 4, 8, 16, 32, 64 and 128 cells. One more palmella division took place to form 256 flagellated, free-swimming dinospores. Later the dinospores settled to the bottom, where they developed into typical peridinian dinoflagellates, the infective form. These dinoflagellates, which are also free swimming, apparently invade the branchial chamber of the fish, become attached to the gill filaments and metamorphose into the parasitic form.

According to Jacobs (1946), *Oodinium ocellatum* is the first dinoflagellate known to parasitize marine vertebrates.

Treatment of Marine *Oodinium* Infestations

The immediate problem was to determine how to relieve the fish of this very prolific parasite and how to eradicate it from the Aquarium's water system. Several methods of treatment were tried; the most effective entailed the use of copper sulfate. That copper is highly toxic to fish and that it must be used with extreme caution is well known. After making a considerable number of tests with tropical marine fishes, I have found that 0.5 p.p.m. is a safe concentration and is lethal to *Oodinium.* When treating large volumes of water, especially in an aquarium where the amount of untreated incoming water may fluctuate greatly, it may be difficult to maintain the concentration exactly at that level. Excellent results can be achieved even though the copper concentration is allowed to fluctuate from 0.4 p.p.m. to 0.8 p.p.m., although 0.8 p.p.m. should be considered the upper limit and should not be maintained for long periods.

Within the allowable range of concentration, the copper induces the fish to secrete a copious

amount of mucus which causes the parasites to become detached. After they are sloughed from the body and settle to the bottom, cell division takes place and development proceeds normally to the free-swimming dinoflagellate stage, and at this point the copper sulfate apparently becomes lethal to them. Since it takes about seven days for the *Oodinium* organisms to develop into free-swimming dinoflagellates, it is necessary to maintain the copper concentration in the tank for at least that long. A ten-day treatment with copper sulfate is recommended.

Nigrelli (1936) reported that numerous marine fishes in the New York Aquarium were at one time heavily infected with *Oodinium ocellatum* and that the infection was not confined to the gills but was found on almost any part of the body. On fishes collected in the vicinity of the Hawaiian Islands I have found *Oodinium* only on the gill filaments. However, in May, 1954, some very sick Clown Fish (*Amphyprion percula*) that had been collected near Singapore were brought to the Aquarium for examination, and both gills and body were found to be covered with *Oodinium*. Treatment with copper sulfate solution was begun immediately. Two days later the parasites had disappeared from the body and gills. These fish were covered with tiny pit marks caused by the parasitic organisms, and one fish, more heavily pitted than the others, died two days later from severe fungus infection apparently resulting from the minute skin punctures. The others lived for several months after treatment, without recurrence of *Oodinium*.

Treatment of Freshwater *Oodinium* Infestations

Oodinium limneticum, a species described by Jacobs (1946), attacks many species of exotic freshwater fishes and causes a malady known to fish fanciers as velvet disease. Jacobs states that this is the first parasitic dinoflagellate known to attack freshwater fishes. It may occur on all external portions of the body, including the fins, trunk, eyes, mouth and gills. A fish parasitized with *O. limneticum* somewhat resembles one with an *Ichthyophthirius* infection, and because of this the disease may be mistakenly diagnosed; microscopic examination is necessary in order to make a positive diagnosis. Treatments most commonly used to cure *Ichthyophthirius* disease usually do not cure fishes with velvet disease.

While this paper was being prepared, velvet disease occurred only once in the Steinhart Aquarium. In this instance a tank of Glassfish (*Chanda lala*) became infected. Copper sulfate was added to the tank at a 0.5 p.p.m. level and two days after treatment was begun the disease disappeared completely. The fish suffered no ill effects from the copper. However, since our experience is so limited, we recommend caution when treating tropical freshwater fish with copper sulfate.

Determination of Concentration of Copper

When treating fish with copper sulfate, it is extremely important to make a daily chemical analysis of the water in the treatment tanks so that a constant level of copper may be maintained. In calculating the amount of copper sulfate needed to make up a solution containing a given amount of the metal, one must take into consideration the fact that the copper represents approximately only 25% of the total weight of $CuSO_4 \cdot 5H_2O$. To determine the amount of $CuSO_4 \cdot 5H_2O$ that must be added to a given volume of water in order to produce a desired concentration of copper in p.p.m., the following equation may be used:

$$x = \frac{v \times p \times 3.93}{1000}.$$

x = weight of $CuSO_4 \cdot 5H_2O$ in grams.
v = volume in liters.
p = parts per million of copper desired.
3.93 = number of grams of $CuSO_4 \cdot 5H_2O$ containing 1 gram of copper.

Copper does not long remain in solution in the presence of excess amounts of carbon dioxide and carbonates; therefore, if practicable, the fish to be treated should be transferred to a clean, nonmetallic tank devoid of all coral, shells, etc. If these substances are present, the copper may react with them to produce insoluble carbonates. Should it not be practicable to remove the fish from such an environment, copper sulfate may have to be added daily to compensate for the loss through precipitation.[1] If freshwater fishes infected with *Oodinium limneticum* are being treated, it is important to know whether the water is hard or soft. Copper is readily precipitated from hard water.

The concentration of copper in water may be determined by a colorimetric method using sodium diethyldithiocarbamate as the indicator. The reagents necessary for this are prepared in the following ways:

Sodium diethyldithiocarbamate solution: Dissolve 1 g of $N(C_2H_5)_2\ CS_2Na$ in 100 ml of copper-free distilled water and keep in a bottle of dark glass protected from sunlight. Add

[1] Precipitation of copper may be substantially decreased by the addition of citric acid to the copper sulfate solution before it is added to sea water. One part by weight of citric acid to 100 parts of copper sulfate usually suffices.

NH_4OH until the pH reaches 9.6-10. This retards decomposition of the carbamate. This reagent will remain stable for approximately 40 days when stored at this pH in a dark place.

Copper-free distilled water: Redistill distilled water, using an all-glass still.

Copper sulfate standard solution: Dissolve 0.393 g of $CuSO_4 \cdot 5H_2O$ in 1 liter of distilled water (copper-free). Dilute 25 ml to 250 ml. One ml of the diluted solution contains 0.01 mg cu.

Ammonium citrate buffer 20%: Dissolve 200 g of citric acid in about 500 ml of copper-free distilled water. Add C.P. NH_4OH until the pH reaches 9.0-9.2. Add copper-free distilled water to 1,000 ml.

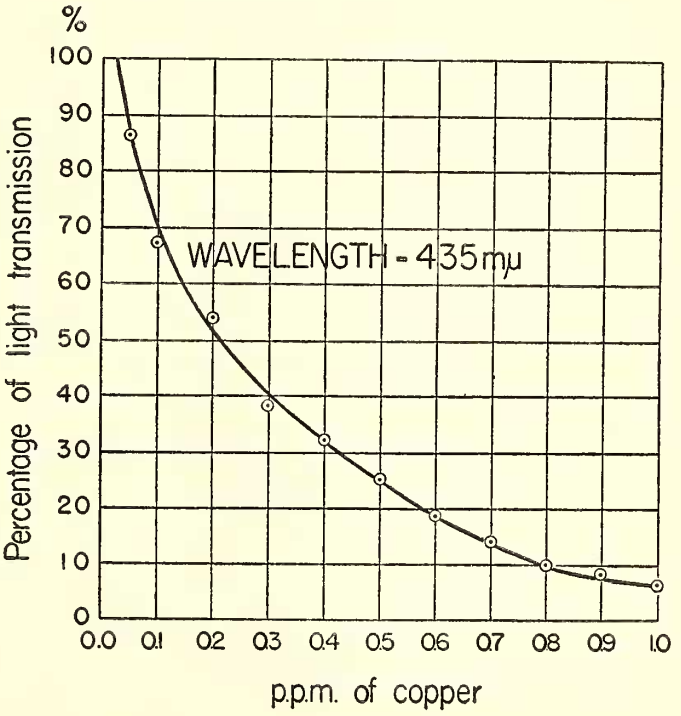
Carbon tetrachloride solution—reagent grade.

The detailed procedure for colorimetric analysis may be described as follows: In a separatory funnel, place 50 ml of the water to be analyzed, 5 ml of ammonium citrate buffer, and mix. Then add 1 ml of sodium diethyldithiocarbamate reagent and mix again. (The carbamate reagent, when introduced into the water sample containing copper, produces an amber color, the intensity of which is in direct proportion to the amount of copper present). To this mixture add exactly 10 ml of carbon tetrachloride and shake for at least 2 minutes to extract the color completely. The solution should be allowed to stand for 10 minutes to achieve complete phase separation. Carefully drain off the colored layer into a test tube and read in the colorimeter.[2] The colorimeter must have been previously adjusted so as to read 100 percent light transmission when a test tube containing the reagent grade carbon tetrachloride has been inserted. To interpret the readings in the colorimeter, it is necessary to refer to a calibration graph based on water samples containing known amounts of copper (Text-fig. 1). These water samples are prepared by adding carefully calculated quantities of the standard copper sulfate solution to 50 ml quantities of copper-free distilled water. The samples are then analyzed in the colorimeter and the readings plotted. Several colored carbon tetrachloride samples were measured at different wave lengths of light, and maximum light absorption was observed at 415 to 450 mu. Table 1 indicates that a wave length of 435 would be ideal. This agrees closely with the findings of Chow & Thompson (1952).

Discussion

It is probable that there is wide variation among different species of fishes in their tolerance to copper. Some tropical marine species begin to show distress at 1 p.p.m. and salmonoid fishes are adversely affected by concentrations

[2] A Bausch & Lomb spectronic twenty colorimeter was used for this purpose.



Text-fig. 1. Calibration graph indicating the percentage of light transmission through samples of known concentration of copper-diethyldithiocarbamate. Samples extracted with carbon tetrachloride from distilled water.

Table 1. Percentage of Light Transmission of Different Wave Lengths of Light through Various Concentrations of Copper-diethyldithiocarbamate Solution. Samples Extracted with Carbon Tetrachloride and Measured with B. & L. Spectronic Twenty Colorimeter.

	Wave Length							
p.p.m. of copper	375 mu	400 mu	415 mu	425 mu	435 mu	450 mu	475 mu	500 mu
.05	97%	92%	88%	87%	86%	89%	94%	98%
.1	95	82	72	69	67	73	85	90
.2	91	75	60	55	54	61	75	88
.3	89	65	46	39	38	46	66	82
.4	87	60	38	32	32	39	61	76
.5	84	53	31	26	25	32	55	73
.6	82	48	25	20	19	24	47	69
.7	81	43	22	15	14	19	42	65
.8	74	36	16	11	10	15	37	58
.9	71	33	13	9	8	12	31	57
1.	70	29	11	7	6	11	30	54

of less than 0.5 p.p.m. Brook Trout fingerlings will die in concentrations above 0.1 p.p.m.

Oodinium disease appears to be very widely distributed throughout the tropic seas and has been reported from aquariums in different parts of the world. The Director of the Taraporevala Aquarium at Bombay reported in 1954 that this gill disease had affected some of the fishes on exhibition there, and the Honolulu Aquarium is periodically invaded by *Oodinium*. Reports of *Oodinium* disease have also come from the Zoological Society of London's Aquarium, the oceanarium (Marine Studios) at Marineland, Florida, and the New York Aquarium. Although this dinoflagellate seems to occur primarily in warm water, it invaded the temperate water system in the Steinhart Aquarium in at least one instance. Shortly after the severe attack on reef fishes in 1951, it was found on some of our local coastal fishes. Several Striped Bass (*Roccus saxatilis*), Rubberlip Seaperch (*Rhacochilus toxotes*) and Lingcod (*Ophiodon elongatus*) died from the disease. The water temperature in their tanks was approximately 65° Fahrenheit at the time of infection. It should also be noted that Nigrelli (1936) found *Oodinium* on fishes collected in Sandy Hook Bay, New Jersey, during the summertime. From this evidence it does not seem unreasonable to believe that this gill disease could invade the cooler waters along the California coast and cause serious damage to an important food fishery.

Summary

At the Steinhart Aquarium, copper sulfate, in concentrations ranging from 0.4 p.p.m. to 0.8 p.p.m., has been found relatively non-toxic for tropical marine fishes, yet effective in eradicating the gill and skin infections of the dinoflagellate, *Oodinium*. Because of the toxicity of higher concentrations of copper, it is essential to control the amount present in the aquarium water. To make this possible a colorimetric method of determining the quantity of copper present in water is described. Velvet disease, which is caused by a freshwater dinoflagellate, *O. limneticum*, also appears to be cured by treatment with copper sulfate.

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